Biologicals xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Diagnostics in the veterinary field: The role in health surveillance and disease identification

ARTICLE INFO

Keywords:
Veterinary diagnostics
Diagnostic kit
Laboratory test
Assay methodology
Disease surveillance
Licensing

ABSTRACT

An international workshop, held in Wiesbaden, Germany on 15–17 May 2019 provided an overview of existing and new methods and approaches to diagnostics in animal health and their benefits and challenges. The variability in quality and authority review of test kits across the world is a concern for the reliability of test results and the decisions that are based on the diagnostic data. In countries or regions without regulatory oversight, there is an urgent need for international harmonisation of quality requirements and licensing procedures. This would increase the validity of the diagnostic methods and allow mutual recognition of test results within the network of official control laboratories and amongst animal health officials. Regional cooperation, as well as the OIE Laboratory Network, should be used to support licensing procedures, pool resources for serum and sample banks, survey outbreak responses, and coordinate research and development of new veterinary diagnostics. The end-users must have clear information on a test's performance, limitations, and interpretation of results.

1. Introduction

Diagnostics are essential tools to ensure the health of domestic and wild animals for both endemic and emerging diseases. They allow animal health professionals to understand and manage the general health status of domestic animals, and they are essential for animal health authorities during the emergence of disease and in the eradication or control of notifiable animal diseases. They are also important tools to support public health initiatives in the case of zoonotic disease control and can be used in the fight against antimicrobial resistance. Diagnostic assays can play an important role in the testing of new and authorised biologics.

To highlight role and importance of diagnostics in animal health, the International Alliance for Biological Standardization (IABS) organized an international scientific workshop on “Diagnostics in the veterinary field: The role in health surveillance and disease identification” in Wiesbaden, Germany on 15–17 May 2019. At the conference, 70 participants from industry, academia, and regulatory bodies discussed the current status and the future goals related to veterinary diagnostics.

Professor H.-J. Bätza from the German Federal Ministry of Food and Agriculture opened the workshop by illustrating various perspectives and issues related to diagnostic testing. A correct diagnosis is the basis for a well targeted animal disease control, and any diagnostic method must be sound and fit for purpose, so the data are sufficiently reliable. Any intervention from a regulatory authority must be based on a trustworthy diagnosis, otherwise there will be a loss of confidence in animal disease control. In case of notifiable diseases, the susceptible animals of a herd may be culled, which means that years of breeding activities are destroyed, where after cleansing and disinfection measures are implemented. In the EU, such measures are often reimbursed in a co-financing between the national authority and the EU Commission. In 2018, the EU Commission allocated around 150 million Euro for the animal health schemes covering e.g. African Swine Fever, Avian Influenza, TSE, rabies, and salmonellae. An authorisation procedure for diagnostic kits is necessary, just as for veterinary drugs and vaccines, and is established in the USA and some EU member states [1], but absent or voluntary in many other regions of the world.

2. Diagnostics: current and new methods (8 experts provided presentations and participated in a roundtable discussion)

Classical tests like Agglutination and Complement Fixation have been used for over 100 years and are still valuable today; e.g., Brucella diagnosis. The more modern serological tests like ELISAs are very commonly used for standard diagnostic work, epidemiological surveillance, and pathogen confirmation. The newer molecular tests like PCR, sequencing, and microarrays are increasingly available and may also be developed to portable Point-of-Care (PoC) methods. Each diagnostic test has related challenges. The specificity and sensitivity may vary between tests and manufacturers, which is important when understanding the limitations of a test, for example where a false-positive or a false-negative test result could have major consequences when found in a disease-free flock or region. Some methods are very labor intensive or require specific skill and equipment, and the cost may differ extensively.

Several examples of new PoC diagnostic equipment were presented and discussed. In a PoC method, the sample, e.g., a nasal swap, is immediately transferred from the animal to the diagnostic device with a few simple handling steps and the result of the analysis is presented directly on the device or a connected computer/smartphone system. A PoC device requires intelligent development because it may be used by untrained people in a non-laboratory environment where complex manipulations are not reasonable. The future goal for new qPCR PoC applications is to ensure a validation of a broad range of veterinary samples, for example swine pathogen panels or bovine mastitis panels.

Diagnostics are crucial in mitigating the effect of disease outbreaks. However, although a broad arsenal of diagnostic methods is available, many of the conventional diagnostic tests are highly specific or targeted

https://doi.org/10.1016/j.biologicals.2019.07.002
Received 24 July 2019; Accepted 26 July 2019
1045-1056/

entirely towards a limited group of infectious agents. Next-generation sequencing (NGS) assays can be developed both for rapid screening of single samples for selected pathogens, and for broad-range pathogen detection and characterisation in multiple samples, including previously unknown organisms. Sequencing-based approaches offer possibilities to identify new and divergent pathogens in diseases with unknown etiology and in multifactorial diseases and co-infections.

Multiplex immunoassay for simultaneous measurement of antibody responses to multiple antigens is a relatively new approach for diagnosing disease outbreaks. It may also be used in vaccine testing to aid a reduction of the number of animals used in potency testing of multivalent vaccines. It has been shown that the method can discriminate between potent and sub-potent vaccines, indicating a potential future use for antibody-based batch potency testing of all vaccine antigens.

Rapid DNA sequencing is developing, and may currently help with outbreak information, management of risk, and identification of new bacteria or subtypes involved in outbreaks. It may also be used to demonstrate antimicrobial resistance (AMR) genes and hence indicate the bacterial susceptibility to specific antibiotics. Given the continuous rise in AMR driven by the correlation between consumption and resistance development, new molecular methods for susceptibility testing that give fast results should be prioritized.

Other innovative diagnostic strategies can also be developed, e.g., sound-monitoring in swine stables to detect increased coughing as an early sign of pulmonary infection.

In summary, the users' expectations are high for the data provided by diagnostic methods, while the quality of validation, the level of information for data interpretation, and the ease of use, may range from evolving to well-established and validated by official laboratories. A possibility to leverage the OIE networks to pool resources was identified, e.g., serum banks from endemic disease regions to be used as control samples in disease-free regions, outbreak response cooperation, and research and development coordination.

3. Diagnostics in epidemiological and clinical surveillance: practical applications (13 experts provided presentations and participated in a roundtable discussion)

The drivers for using diagnostics in practical veterinary settings for surveillance, control and eradication programs are i) human and animal health and welfare, ii) food safety, and iii) economics. It is essential to accept that ALL diagnostic tests are imperfect, and to recognize the limitations of the test and adopt flexible strategies. No cut-off values suit all programmes; interpretation level needs to be adapted in the presence of herd infection versus negative herds.

The use of DIVA (Differentiating Infected from Vaccinated Animals) DNA vaccines in combination with the accompanying marker diagnostic test systems has proven to be a very efficient tool in control and eradication of Pseudorabies virus (Aujeszky's disease) and Bovine Herpesvirus type-1 (IBR/IPV), two economically important diseases in livestock in Germany. However, limitations of the diagnostic marker tests make it crucial to control the epidemiological plausibility of the results by the veterinary authorities. The success of eradication programs is dependent on efficient diagnostics, consistent selection of infected animals, stringent vaccination schedules, professional farm management, effective hygiene- and quarantine measures, and fair reimbursement of losses.

An example from bovine tuberculosis eradication illustrated how important it is to identify confounders impacting on test performance [2]: The quality of the tuberculin used in the test, was not monitored against an ISO standard nor were there external laboratory Ring Trials to ensure reagent conformed to set specifications, resulting in extensive variability between tuberculin brands. Such poor quality of tuberculin used in the tests may lead to a failed surveillance program and persistence of the disease despite eradication efforts.

The prevalence of Transmissible Spongiform Encephalopathies (TSE) in the EU has decreased substantially over the last decades due to intense surveillance and diagnostic efforts, which have evolved from a 3-week histology test over serological methods to a 1-day rapid test today.

In the surveillance of pancreatic disease (SAV) in salmon, it is essential to define if the test must confirm clinical cases, establish early detection of infection and disease outbreaks, or demonstrate freedom from disease in a population. Depending on where in the evolution of the disease the sample is taken, some diagnostic tests are more suitable than others. Best practice should include a combination of diagnostic tools. Serology is an underused and misunderstood diagnostic tool in fish medicine, in contrast to the widespread and successful use in terrestrial animals.

The highly topical disease, African Swine Fever (ASF) is a very contagious, hemorrhagic disease of pigs caused by a complex virus with a complex epidemiology depending on the geographical distribution – and for which no vaccine so far is available. It has been endemic in sub-Saharan Africa for decades but recently jumped to eastern Europe and Asia, where it spread rapidly. Validated ASF diagnostic techniques are available for giving a confident diagnosis of ASF in the affected countries. However, combining both ASF virus and antibody detection improve the efficacy of disease-control measures.

A survival study of viral pathogens in animal feed ingredients under transboundary shipping models [3] demonstrated the potential for environmental contamination of animal feed was very large and that many infectious virions survived in different feed matrixes and through long transport conditions. In a specific sampling situation, 1–2% of feed ingredient samples could be shown positive for ASF virus DNA, as well as feed mills, trucks, personnel equipment, and other fomites. Viral survival was also demonstrated throughout extended transit times. Feed is now a well-recognized vehicle for the transport and transmission of pathogens at the domestic and global level.

A new way of re-using old tissue samples was demonstrated for Newcastle disease; formalin-fixed paraffin-embedded bird tissues in historical samples were used successfully for unbiased Next-Generation Sequencing (NGS), in epidemiological or evolutionary studies.

The need to constantly monitor vaccines and field isolates through adequate diagnostic tools, and the need to develop DIVA vaccines, was exemplified with Lumpy Skin Disease, for which control is only effective through vaccination.

In practical bovine health care, rapid PoC tests have been developed to diagnose calf diarrhea enteropathogens and to establish the level of maternally derived antibodies transmitted to a calf.

"Big Data" in animal health can combine production data with tracking, feed intake, milk quality, reproduction data, and weather conditions, to give early warnings of disease occurrence or other emerging problems. However, many veterinary schools have not prepared students for using these technologies.

4. Acceptance of data, qualification tools for laboratories and methods (9 experts provided presentations and participated in a roundtable discussion)

The development of a diagnostic assay may take several years, excluding the basic research and development of equipment. Many technical, economic and regulatory challenges must be dealt with, and there may be a disconnect between the wishes in performances and the acceptable price. A harmonisation of the regulatory process would be beneficial for assay manufacturers, because multiple national agencies, multiple dossier formats, and multiple (sometimes contradictory) requirements are obstacles that could be removed.

It is ideal for the development of analytic and diagnostic assays in a biological product development to run in parallel with the development of new vaccines. Specifically developed and validated assays are needed to identify antigens, for in-process control tests, and to measure activity, efficacy, stability and potency of the vaccine.

In the USA, several laboratory networks cooperate on routine and emergency responses, to identify novel and emerging microbial strains, and to ensure testing capacity. The American Association of Veterinary Laboratory Diagnosticians (AAVLD) manage an accreditation program, which is a comprehensive laboratory evaluation based on specific requirements and the laboratory's quality management system (QMS). Other laboratory networks exist and use different validation systems. The USDA licensing system authorizes the manufacture of diagnostic test kits. This system requires controlled production of kit components, to minimize variation within and between batches. The USDA evaluates kits before and after licensure to ensure compliance with production and testing standards [4].

In the EU, the Official Medicines Control Laboratories (OMCL) serve as a public institution performing testing of products on behalf of a Competent Authority, including quality assurance of laboratories and methods [5]. All tests are performed under ISO standard 17025: *General requirements for the competence of testing and calibration laboratories*. For veterinary diagnostics, only some EU member states, e.g., Germany, have a licensing procedure. A harmonized and common licensing of veterinary diagnostics would provide a more reliable diagnosis of major diseases, which is of paramount importance for animal disease control and has major impact on several sectors. The German authorisation procedure comprises submission of an application, an evaluation of documents, labels and instructions, and an experimental testing in the respective test laboratory of the Friedrich-Loeffler-Institute, prior to authorisation. To increase the validity of veterinary diagnostics in the EU in general, the establishment of a similar and EU-harmonized system would be beneficial.

On a global level, the OIE has implemented a procedure for registration of diagnostic kits [6]. The aim is to certify a kit as validated fit for specific purpose(s) and to produce an OIE register of recognized diagnostic kits (available on the OIE website). So far, 11 diagnostic kits are included in the register, but once a kit/method is adopted by the general assembly, it is applicable in all 182 OIE Member Countries.

The OIE and FAO laboratories undertake the dissemination of protocols/SOPs, practical training and inter-laboratory proficiency-testing to demonstrate intra/inter-laboratory equivalence. This is an important tool to support, because it monitors the development of capacity in laboratories. However, the proficiency tests exercises are expensive and time consuming, so e-learning courses are being developed as an additional tool.

In the EU, participation in inter-laboratory proficiency test is mandatory as part of the National Reference Laboratories' accreditation and is useful for harmonisation and standardization of laboratory tests. The EU Commission sustains this important but laborious activity.

5. Conclusions and recommendations

The following conclusions and recommendations were developed in the final session of the Workshop and were refined by attendees in the first two weeks after the event.

5.1. Conclusions

5.1.1. Diagnostic tests are performed for

- Routine surveillance of health or immunological status including freedom from disease.
- Diagnosis of emerging and other diseases to support intervention decisions.
- Disease eradication initiatives including vaccine/DIVA strategies and trade restrictions.
- Testing food, feed, and the environment for pathogens and for antimicrobial resistance genes.
- Testing of vaccines for performance, potency, and quality.

The current quality spectrum of tests is evolving, especially with the application of new technology, with the validation differing from test to test, except kits under the control of a National Regulatory Authority.

5.1.2. Test methods

- In addition to "classical" tests which have and will continue to have their place in diagnosis of all kinds of health surveillance, new methods come in. The value of classical serologic tests, some of which have been in use for more than 100 years is recognized, even if the number and capabilities of new molecular tests is broad and growing. As always, greater confidence regarding test interpretation comes with greater experience in using the methodology under diverse real-life situations.
- Newer molecular diagnostic tests can be extremely valuable tools in animal health systems, especially in the ability to test for multiple pathogens (multiplexing) and improving testing result timelines. Rapid tests can serve as early warning tools and free time to enhance biosecurity and vaccinate to contain an outbreak.
- Sequencing is an excellent tool for molecular epidemiology and provides easy availability of big data. Real understanding of the data, supplemented by field observations and disease agent characterisation, provides a good opportunity to identify epidemiological threats.

5.1.3. Use of diagnostics

- There is a permanent challenge with having the right sample/specimen sent in the right way to laboratories – this shows the need for better communication to vets and farmers, and better education of vet students in diagnostics.
- For frontline use, vets and farmers need support to analyse these data quickly and easily to allow immediate decisions on further action.
- Disease prevalence, clinical relevance, and "fitness for purpose" need to be considered when choosing a diagnostic test, especially for emerging and notifiable diseases. Understanding the test expectations and limitations (e.g. false positives and false negatives test results) is critical when interpreting results, in particular for trade and animal movement.
- Cost/affordability of individual tests is a significant factor in the use of the diagnostic methodologies.
- Development of DIVA vaccines and diagnostics simultaneously is ideal, but not always realistic. However, DIVA strategies are requested by the public and politicians more and more, because stamping-out strategies are not perceived as acceptable anymore.
- Confirming results for the diagnosis of controlled (notifiable) diseases via a National Authority Laboratory remains a critical step in the diagnostic process and must serve as the basis for decisions on disease management, e.g. culling/reimbursement.

5.1.4. Official approval/licensing

- Diagnostic tests must be "safe" and sound, so we can rely on the findings. A consistent approval process by authorities will serve this goal. Currently, the lack of EU approval is compensated by the cooperation and validation performed by many reference laboratories (EU and national). In USA licensing is established. Technical expectations for authorisation data vary by country, complicating test development and approval.
- Proficiency testing of reagents are not enforced/applied. There may be a huge variability in potency of reagents even in marketed products that claimed to conform to OIE requirements, which could have a disastrous effect on a disease control program and lead to a general mistrust of test results.
- The variability of the regulatory scrutiny of veterinary diagnostic

tests across the world is very large, ranging from strict authority licensing and control to no official regulation at all. This may impact on international trade due to variable requirements.

5.2. Recommendations

5.2.1. Official approval/licensing

- There is an urgent need for international harmonisation of quality requirements and licensing procedures. Setting of common standards on the quality of manufacture and validation of test systems is needed.
- Propose that the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Products (VICH) [7] consider asking to extend their mandate by laying down technical requirements for diagnostics. This should take into consideration the already existing OIE relevant Chapters of the Manuals (1.1.6 of the Terrestrial and 1.1.2 of the Aquatic Manual) [8,9].
- Establish licensing procedures to ensure a defined level of quality, validation, storage and transport conditions and follow up procedures to evaluate the performance of test systems in the field. The authorities should take steps to ensure that approval/license is withdrawn when license conditions, stipulations and label claims are not met, and that such products are taken off the market.
- Compliance with internationally agreed upon OIE diagnostic methods (Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [8] and Manual of Diagnostic Tests for Aquatic Animals [9]) is essential. Proof of compliance needs to be provided by the relevant labs.
- The OIE network should be used to pool resources for serum and sample banks, outbreak response, and R&D coordination. Feedback should be sent back to OIE-reference labs.
- For the EU, a common licensing procedure should be established, which ensures the possibility for quick reactions to immediate threats. This also applies to other regions without a licensing procedure.
- Establish official written and physical standards for calibration of test systems. This will allow the mutual recognition of test results within the network of official control labs and ensure the use of identical test kits within the regions where tested animals are moved.
- Clear information should be available for the test user/buyer on the test's performance, limitations, and interpretation of results.

5.2.2. Quality of diagnostics

- Test Validation (sensitivity, specificity, repeatability, target population) is critical as is manufacturing quality control and oversight by Regulatory Authorities. Distinguish peace time and emergency licensing, and tailor the regulatory scrutiny to the risk; endemic, exotic, and notifiable diseases.
- Until an authorisation procedure is established, the growing number of new pen-side tests for pathogen detection and serology should be validated through the established networks of reference laboratories and their partners.

5.2.3. Use of diagnostics

- Establish a system of quick data transfer from the field to official control labs for verification, in particular when notifiable diseases are concerned.
- Multi-parameter testing, such as a battery of test methodologies; or point-of-care versus in-laboratory tests, has the potential to improve diagnostic certainty.
- In the Diagnostic Industry *"Big Data"* are upcoming, and standardized processes to handle, secure and interpret these are needed.
- When considering the risks associated with transboundary diseases, testing samples other than animal samples such as fomites and the environment need to be considered. Newer information indicates that feed may be a significant risk for spreading of pathogens.

Declarations of interest by the authors

None.

Acknowledgements

The authors would like to thank the chairs of the workshop for their excellent guidance through the sessions, the speakers for their valuable presentations, and the participants for their constructive contributions to the discussions. The presentations and full text of conclusions and recommendations can be found on the IABS website [10], members section.

Speakers who agreed to the publication of their name and affiliation:

Claudio Afonso, US National Poultry Research Center, USA.

Neil Almond, National Institute for Biological Standards and Control (NIBSC), UK.

Keith Bailey, American Association of Veterinary Laboratory Diagnosticians (AAVLD)/Oklahoma Animal Disease Diagnostic Laboratory (OADDL), USA.

Belen Barreiro, Ingenasa, Spain.

Hans-Joachim Bätza, Federal Ministry of Food & Agriculture, Germany.

Sandra Blome, Friedrich Löffler Institut, Germany.

Elena Maria Bozzetta, Istituto Zooprofilattico Sperimentale Del Piemonte Liguria E Valle d'Aosta, Italy.

Scott Dee, Pipestone Veterinary Services, USA.

Carmina Gallardo, Centro de investigación en sanidad animal, Spain.

Margaret Good, Private Consultant, Ireland.

Fredric Granberg, Swedish University of Agricultural Sciences, Sweden.

Timm Harder, Friedrich-Loeffler-Institut, Germany.

Estelle Hess, Bio-X Diagnostics, Belgium.

Marianna Ivok, CEVA-Phylaxia Veterinary Biologicals Co. Ltd.; Hungary.

Donald King, OIE/FAO Reference Laboratory for Foot-and-Mouth Disease, UK.

Serge Leterme, IDEXX, USA.

Larry Ludemann, Center for Veterinary Biologics, USDA, USA.

Hege Lund, Norwegian University of Life Science, Norway.

Marc Marenda, The University of Melbourne, Australia.

Marian McLoughlin, Fish Vet Group, UK.

Gleeson Murphy, National Veterinary Services Laboratories, USA.

Niels Jorgen Olesen, DTU Aqua, Denmark.

Heinz Schoeder, Boehringer Ingelheim VRC GmbH & Co KG, Germany.

Carsten Schroeder, INDICAL BIOSCIENCE GmbH, Germany.

James Stanton, University of Georgia, USA.

Carlos Suarez, Animal Disease Research Unit, Agricultural Research Service, USDA, USA.

Maria Szabo, OIE World Organisation for Animal Health, France.

Alex Van Belkum, bioMerieux, France.

David Wallace, ARC-Onderstepoort Veterinary Research institute, South Africa.

William Wilson, U.S. Department of Agriculture, USA.

References

[1] Gesetz zur Vorbeugung vor und Bekämpfung von Tierseuchen (German law on Animal Health diagnostics section http://extwprlegs1.fao.org/docs/pdf/ger124332.

pdf, Accessed date: 10 July 2019 11, 2.
[2] Bakker, D. and Good, M. (2018). Quality control of Purified Protein Derivative tuberculins: essential for effective bovine tuberculosis control and eradication programmes. ISVEE, 12-16 november 2018 Thailand.
[3] Dee SA, et al. Survival of viral pathogens in animal feed ingredients under transboundary shipping models. https://doi.org/10.1371/journal.pone.0194509; March 20, 2018.
[4] USDA website on animal health and diagnostics. https://www.aphis.usda.gov/aphis/ourfocus/animalhealth, Accessed date: 10 July 2019.
[5] OMCL website. https://www.edqm.eu/en/general-european-omcl-network-geon, Accessed date: 10 July 2019.
[6] OIE-website. Registration of diagnostic kits. www.oie.int/scientific-expertise/registration-of-diagnostic-kits/background-information/, Accessed date: 10 July 2019.
[7] VICH website. The international cooperation on harmonisation of technical requirements for registration of veterinary medicinal products. https://vichsec.org/en/, Accessed date: 10 July 2019.
[8] OIE manual of diagnostic tests and vaccines for terrestrial animals. http://www.oie.int/en/standard-setting/terrestrial-manual/access-online/, Accessed date: 10 July 2019.
[9] OIE manual of diagnostic tests for aquatic animals. http://www.oie.int/en/standard-setting/aquatic-manual/access-online/, Accessed date: 10 July 2019.
[10] IABS website. International alliance for biological standardization. https://www.iabs.org/, Accessed date: 10 July 2019.

Anja Holm
Central VetPharma Consultancy, Hauchsvej 7, 4180, Soroe, Denmark

Richard Hill
International Alliance for Biological Standardization, USA

Attila Farsang
Ceva-Phylaxia Co. Ltd., Hungary

Carmen Jungbäck*
International Alliance for Biological Standardization, Germany
E-mail address: carmen.jungbaeck@iabs.org.

* Corresponding author. IABS, Rue de la Vallé 3, 1204, Geneva, Switzerland. Tel.: +49 69 555944.